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US Army Corps of Engineers
Waterways Experiment Station

Long-Term Effects of Dredging Operations Program

Quality Assurance/Quality Control (QA/QC) Guidance for Laboratory Dredged Material Bioassays

Results of QA/QC Workshop Held May 26-27, 1993, in Seattle, Washington

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Battelle/Marine Sciences Laboratory

Prepared for Headquarters, U.S. Army Corps of Engineers

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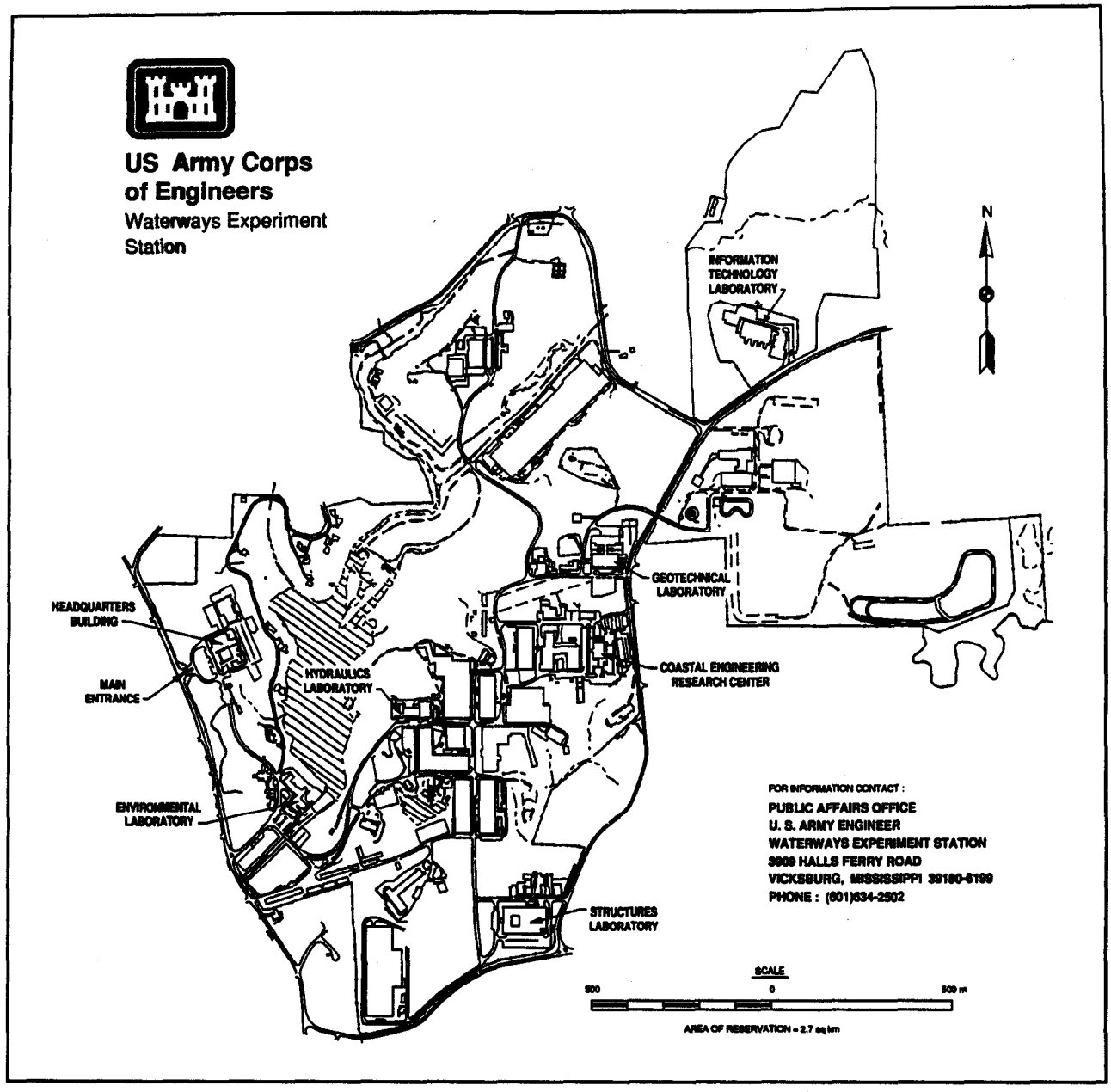

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Contents

Preface

The report herein was prepared following a quality assurance/quality control workshop conducted to establish guidelines for laboratory dredged material bioassays. The workshop was sponsored by the U.S. Army Engineer Waterways Experiment Station (WES), coordinated by the Battelle/Marine Sciences Laboratory (MSL),[1] and conducted at Battelle's Human Affairs Research Center in Seattle, WA, on May 26-27, 1993.

This report was prepared by Drs. David W. Moore and Thomas M. Dillon, Fate and Effects Branch (FEB), Environmental Processes and Effects Division (EPED), Environmental Laboratory (EL), WES, and Dr. Jack Q. Word and Mr. Jeffrey A. Ward, MSL. Thanks are extended to Ms. Kristen Hiraoka of the Battelle Human Affairs Research Center for arranging the meeting rooms and accommodations in support of this workshop, and to Mr. Tim Thompson for providing examples of contractual documents and testing forms used in this document.

The report was prepared under the general supervision of Dr. Bobby L. Folsom, Jr., Chief, FEB; Mr. Donald L. Robey, Chief, EPED; and Dr. John Harrison, Director, EL.

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[1] The Battelle/Marine Sciences Laboratory is part of the Pacific Northwest Laboratory, which is operated for the U.S. Department of Energy by Battelle Memorial Institute.

1 Introduction

Currently, there is little established guidance pertaining to Quality Assurance/Quality Control (QA/QC) in the toxicological testing of dredged material programs conducted by the U.S. Army Corps of Engineers (USACE). Two guidance documents are presently used to evaluate dredged sediment: "Evaluation of Dredged Material Proposed for Ocean Disposal - Testing Manual" (*Implementation Manual* or Green Book) and the draft "Evaluation of Dredged Material Proposed for Discharge in Inland and Near Coastal Waters - Testing Manual" (*Inland Testing Manual*). During fiscal year 1994, the U.S. Environmental Protection Agency (USEPA) will provide detailed information concerning QA/QC for individual sediment bioassays used in the regulatory evaluation of dredged material. This information will enhance the ability of District personnel to develop appropriate QA/QC plans and data quality objectives.

To develop more generic guidance, the U.S. Army Engineer Waterways Experiment Station (WES) and the Battelle/Marine Sciences Laboratory (MSL) coordinated a workshop that drew upon the expertise of individuals who conduct laboratory toxicity assessments and/or are responsible for QA/QC issues. (A list of workshop attendees is included in Appendix A.) Results of this workshop were used to develop this generic guidance document. This document describes suggested QA/QC procedures relating to the following issues:

a. Data quality objectives (DQOs).

b. Biological procedures.

c. Sample handling, storage, and shipment.

d. Data recording, reduction, validation, and reporting.

e. Internal quality control checks.

f. Corrective action.

Also included is a section on other related issues raised during the workshop.

QA/QC is an essential element of dredged material evaluation projects. The QA/QC program provides the framework for assessing the quality of data associated with a dredged material program and ultimately determining whether the data generated during the study is of sufficient quality to be used in decision making. Programs with an adequate QA/QC program have a clear structure of responsibility, formal data quality objectives, defined procedures and protocols for testing, and a mechanism for identifying and correcting potential problems. This enables project managers to assess the quality of data for decision-making purposes. In contrast, programs with substandard QA/QC often have no way of assessing the quality of data. Conflicts associated with suspect data may not be resolved, and the decision-making process is either slowed or stopped completely. Thus, a comprehensive QA/QC program is fundamental to the success of a project.

Quality assurance management plans (QAMPs) vary in content depending on program needs, but should address the following elements:

a. A description of the program organization and responsibilities.

b. Definition of data quality objectives.

c. Sampling procedures.

d. Instrument calibration procedures.

e. Procedures for recording, reducing, validating, and reporting data.

f. Procedures for performing quality assurance verification and internal quality control checks.

g. Preventative maintenance schedules.

h. Specific routine procedures to evaluate precision, accuracy, and completeness.

i. Definitions of deviations and appropriate corrective actions.

j. Information on appropriate indoctrination and training.

2 Data Quality Objectives

DQOs for evaluating dredged material toxicity must be established to ensure that information obtained will provide an accurate and precise estimate of predicted environmental effects. These DQOs identify (a) the appropriate accuracy and precision of measurements and (b) the types of measurements to be made. These two criteria determine the level of quality required for toxicity tests that ultimately establishes whether or not dredged sediment is acceptable for open water disposal. For example, DQOs associated with a typical toxicity test may require that reference toxicant and test validation controls be run concurrently with the dredged sediment evaluation to assess test organism sensitivity and health. The DQOs will also define the acceptable range of response for these exposures. District personnel should attempt to establish appropriate DQOs that allow for the evaluation of test performance relative to the question to be answered and avoid establishing DQOs that do not enhance these evaluations.

During the QA/QC Workshop, discussion of DQOs centered on three issues:

a. Federal guidance concerning DQOs.

b. Statistical sensitivity and power.

c. Required DQOs.

Federal Guidance Concerning Data Quality Objectives

Federal guidance for marine and estuarine waters contained in "Title 40, Code of Federal Regulations (CFR)," Parts 220-228, and that provided in the joint USEPA/USACE document "Evaluation of Dredged Material Proposed for Ocean Disposal - Testing Manual" (*Implementation Manual*, EPA-503/8-91/001) (USEPA/USACE 1991) were reviewed and summarized as follows:

> Section 227.13(b) Dredged material which meets the criteria...is environmentally acceptable for ocean dumping without further testing...*When*...[Section 227.13(c)(3)]

> Bioassays on the suspended particulate and solid phases show that it can be discharged so as not to exceed the limiting permissible concentration as defined in paragraph (b) of Section 227.27 [LPC].

Subpart G, Definitions, defines the limiting permissible concentration (LPC) as follows:

> Section 227.27(3)(b) The limiting permissible concentration of the suspended particulate and solid phases of a material means that concentration which will not cause unreasonable acute or chronic toxicity or other sublethal adverse effects based on bioassay results using appropriate sensitive marine organisms.

Federal guidance for inland and near-coastal waters relative to 33 CFR 320-330 and 40 CFR 230 under Section 404 (permitting) and the guidance provided in the joint USEPA/USACE document "Evaluation of Dredged Material Proposed for Discharge in Inland and Near-Coastal Waters - Testing Manual (Draft), Inland Testing Manual" were also evaluated. Proposed discharges of dredged material must comply with 40 CFR 230, Section 230.10:

> Section 230.10(c) requires that discharge of dredged material not result in significant degradation of the aquatic ecosystem.

Suspended-particulate-phase and solid-phase toxicity tests are used to assess environmental impacts in support of these requirements as directed by Section 230.61.

Therefore, the objective of dredged material toxicity testing is to determine whether or not the material is suitable for open water disposal under Titles 33 and 40 CFR. As a result, DQOs must be written to ensure that the presence or absence of unacceptable toxicity can be determined. Once these DQOs are clearly stated, contracts and indemnification statements can be developed to protect both the contractor and the client. Examples of these contracts are presented in Appendix B.

Statistical Sensitivity and Power

The *Implementation Manual* and *Inland Testing Manual* were then reviewed to determine a precise statement on the required statistical sensitivity and power in support of toxicological testing programs. The following are criteria used in the manuals to make this determination:

Elutriate phase *(Implementation Manual)*

a. **Acutely toxic concentrations.** Acutely toxic concentrations of the dissolved and suspended contaminants released from sediment during the preparation of the elutriate phase are evaluated by determining (a) whether a statistically significant increase in mortality or sublethal

effects occurred between the highest concentration of the elutriate and a clean seawater control, (b) whether the effect was sufficiently large to produce a 50-percent change in the response, (c) what concentration of elutriate will produce that effect, and (d) whether a model predicts that concentrations of elutriate above 0.01 will be present beyond the disposal site any time or within the disposal site after 4 hr of initial mixing.

b. **Determination of compliance.** The *Implementation Manual* (6.1) indicates that dredged material does not meet the LPC for the elutriate phase if the concentrations of the dissolved and suspended contaminants exceeds 0.01 of the acutely toxic concentration (LC_{50}) beyond the disposal site at any time and/or within the disposal site after the 4-hr initial-mixing period.

Elutriate phase (Inland Testing Manual)

a. **Acutely toxic concentrations.** According to the *Inland Testing Manual*, acutely toxic concentrations of the dissolved and suspended contaminants released from sediment during the preparation of the elutriate are evaluated statistically if survival in the dilution water treatment is at least 10-percent greater than the 100-percent elutriate treatment. The LC_{50} response for each sediment treatment is then calculated. If a statistically significant difference exists between elutriate concentrations and the dilution water, and if greater than 50-percent mortality or other effects occur in all treatments, it is not possible to calculate an EC- or LC_{50}. In a situation where it is not possible to calculate an LC_{50}, the 100-percent elutriate is used as a conservative estimate of the LC_{50} for input to the mixing zone model. If conditions are highly toxic, such that the 10-percent concentration has a greater than 50-percent mortality, further dilution must be made (using new treatments with less than 10-percent elutriate concentrations).

b. **Determination of compliance.** If the 100-percent elutriate treatment is not statistically different from the dilution water using a two-sample t-test, the elutriate is predicted not to be acutely toxic to water column organisms. If an LC_{50} can be calculated, the modeled concentrations of the dredged material (expressed as percentages) are compared with 0.01 of the 48- or 96-hr LC_{50}, depending upon the test duration. The maximum allowable concentration outside the mixing zone is 0.01 of LC_{50}.

Solid phase *(Implementation Manual)*

a. **Acutely toxic concentrations.** During solid-phase toxicity testing, evaluations are made using one-tailed, 95-percent confidence limits to determine statistical significance relative to a reference sediment. The power of the test assuming the true standard deviation is 5 percent

(allowable mortality in native control samples cannot exceed 10 percent; most testing shows the standard deviation to be less than half the allowable range of native control toxicity) and the size of the difference beyond the standard deviation is either 10 or 20 percent ($n = 5$, $\alpha = 0.05$).

b. **Determination of compliance.** The *Implementation Manual* (6.2) indicates that dredged material does not meet the LPC for solid-phase testing if the mortality of the test organisms is (a) statistically significantly (statistically) greater than in the reference sediment treatment and (b) the mortality exceeds the reference sediment by at least 10 percent (or a value that is in accordance with approved testing methods, e.g., 20 percent for amphipod tests).

Solid phase *(Inland Testing Manual)*

a. **Acutely toxic concentrations.** The *Inland Testing Manual* indicates that if survival in dredged material is higher than or equal to the survival observed in the reference or control sediment, then no statistical analysis is needed and the dredged material shows no indication of causing adverse environmental effects. However, if survival in the reference material is higher than that in the dredged material treatments and exceeds the allowable percent difference between the two treatments, then the data has to be statistically analyzed to determine whether there is a significant difference between the reference and dredged material. Evaluations of solid-phase results are made using Fisher's Least Significant Difference (LSD) when the survival or other response of two sample means is being compared. The LSD is usually performed following with analysis of variance (ANOVA). When parametric tests are not appropriate for multiple comparisons because the normality assumption is violated, nonparametric procedures should be employed. The assumptions of statistical power are similar to those discussed for the *Implementation Manual.*

b. **Determination of compliance.** According to the *Inland Testing Manual,* dredged material is predicted to be acutely toxic to benthic organisms when test organism mortality is statistically greater in the dredged material than in the reference sediment and exceeds reference mortality by at least 10 percent (20 percent in amphipods).

The degree of required sensitivity and precision has therefore been established based upon the above determinations. Alteration in laboratory toxicity results introduced into data evaluations that occur after meeting all DQOs must not reduce the precision and power of the tests as presented in the *Implementation Manual* and the *Inland Testing Manual.* For solid-phase tests, it appears that this means that the probability of a type 1 error (rejection of the null hypothesis when it is in fact true) must remain at <0.05. Guidance is not available for the appropriate rejection levels for elutriate tests.

Required Data Quality Objectives

The following categories of observations require DQOs, given the testing objectives described in the *Implementation Manual* and given the statistical power required:

a. Water quality (temperature, salinity, hardness, dissolved oxygen, alkalinity, ammonia, etc.).

b. Minimum survival in native control sediment.

c. Sensitivity of test organisms (reference toxicant effects).

d. Interlaboratory and intralaboratory performance standards.

e. Frequency of observations.

f. Number of replicates.

3 Biological Procedures

Biological procedures are the written protocols or instructions describing how to perform all routine measurement activities associated with toxicological testing and related QA/QC activities. These procedures must be followed to ensure the integrity and quality of data. During the QA/QC Workshop, four major issues were discussed:

a. Standard operating procedures (SOPs) and checklists.

b. Good laboratory practices.

c. Statistical design and randomization.

d. Choice of appropriate tests.

Workshop participants universally agreed that two of the most important QA/QC issues relating to biological procedures were the interlaboratory and intralaboratory standardization in the conduct of toxicological tests. Suggested lists of test species for freshwater and marine testing are presented in the *Inland Testing Manual* and *Implementation Manual.* Other species may be substituted depending on program needs and regional guidance.

Standard Operating Procedures (SOPs) and Checklists

One way to ensure consistency of toxicological testing and reporting is for Districts to require the use of SOPs and standardized data forms by contractors. Appendix C contains examples of quality control checklists, project schedule lists, procedural checklists, test and reference toxicant procedures, setup forms, daily observation and monitoring forms, and test termination forms. It was suggested at the QA/QC Workshop that laboratory SOPs should be written for all routine or repetitive activities and periodically reviewed and updated as necessary. District personnel are advised to determine whether potential contractors have the appropriate standard procedures available prior to finalizing a contract.

Good Laboratory Practices

Good laboratory practices should include both blind testing to eliminate analyst bias and randomized block designs to eliminate treatment effects related to test chamber position. The completely randomized block is the simplest form of design in which treatments are allocated to the experimental units (aquaria or test jars, for instance) at random. That is, every unit has an equal chance of receiving a particular treatment. In addition, the units are processed in a random order at all subsequent stages of an experiment where this order is likely to affect the results of a test. For example, test containers that are maintained with water or light should be randomly positioned within the testing area. If all replicates from a single treatment are placed together, then it is no longer clear if treatment differences are associated with the treatment alone or with their position within the test environment. Test containers should be analyzed in a random order and in the blind (the treatment is unknown to the observer). This prevents biases associated with increased skill at taking measurements or knowledge of test-treatment identity.

Testing methods should also minimize the potential of cross-contamination by volatiles, and standardized reference toxicants should be run whenever possible. For marine toxicological tests, cadmium is used extensively as a reference toxicant, but poses disposal problems. Potassium Chloride (KCl) is often used in freshwater systems, as are copper and zinc. Workshop participants advised that a reference toxicant should be used that can be compared with an established database.

Control charts are used to assess whether the sensitivity of test organisms to a given reference toxicant is within a predetermined range of acceptability. A control chart is constructed by plotting successive toxicity values (for example, LC_{50}'s) for a given reference toxicant and determining the cumulative trends exhibited for this series of samples. The mean and standard deviation are recalculated with each successive plot until the statistics stabilize. Outliers, which are values that fall outside the upper and lower limits, are readily identifiable.

Testing procedures, including acclimation, test conduct, laboratory controls, statistical design, and randomization, are generally provided in test protocols and are based, in part, on project objectives. Suggested minimum requirements for test monitoring are presented in Tables 1 and 2.

Statistical Design and Randomization

It was agreed at the QA/QC Workshop that, if other guidance is not available, the appropriate statistical design for toxicological tests should include, at a minimum, five replicates for test treatments and one to three replicates for reference toxicant exposures. Both blind testing and a randomized block design should be employed to reduce position effects. A power analysis

Table 1
Suggested Monitoring Requirements for Solid-Phase Tests

Parameter	Frequency of Measurements		Extent
	Interstitial Water	Overlying Water	
Dissolved Oxygen		Daily	One replicate/ treatment
Temperature		Daily	At least five locations in test array
Alkalinity[1]	Beginning	Beginning/End [2]	One replicate/treatment
Hardness[1]	Beginning	Beginning/End [2]	One replicate/treatment
Conductivity[1]	Beginning	Beginning/End [2]	One replicate/treatment
Ammonia	Beginning	Beginning/End [2]	One replicate/treatment
pH	Beginning	Beginning/End [2]	One replicate/treatment
Salinity[3]	Beginning	Daily	One replicate/treatment
Total Sulfides	Beginning	Beginning/End [2]	One replicate/treatment
Flow Rates[4]			One replicate/treatment

[1] Freshwater tests only.
[2] Or prior to water exchanges during renewal tests.
[3] Marine/estuarine tests only.
[4] If applicable, measured daily.

should be performed prior to increasing levels of replication since additional replicates may not necessarily enhance sensitivity relative to project needs nor justify the associated cost increases.

Other Related Issues

Toxicological testing is usually accompanied by physical/chemical evaluation of tested sediments. Physical/chemical parameters that may be measured include the following:

Polynuclear Aromatic Hydrocarbons (PAHs)
Polychlorinated Biphenyls (PCBs)
Pesticides
Phenols
Phthalates
Metals
Butyltins
Dioxins/Furans
Grain Size/Total Solids
Atterburg Limits

Table 2
Suggested Monitoring Requirements for Elutriate Tests

Parameter	Frequency	Extent of Measurements
Dissolved Oxygen	Daily	One replicate/concentration/ treatment
Temperature	Daily	At least five locations in test array
Alkalinity[1]	Beginning/End [2]	One replicate/concentration/ treatment
Hardness[1]	Beginning/End [2]	One replicate/concentration/ treatment
Conductivity[1]	Beginning/End [2]	One replicate/concentration/ treatment
Ammonia	Beginning/End [2]	One replicate/concentration/ treatment
pH	Beginning/End [2]	One replicate/concentration/ treatment
Salinity[3]	Daily	One replicate/concentration/ treatment
Total Sulfides	Beginning/End [2]	One replicate/concentration/ treatment

[1] Freshwater tests only.
[2] Or prior to water exchanges during renewal tests.
[3] Marine/estuarine tests only.

Settling Rates
Total Volatile Solids
Total Organic Carbon (TOC)
Cyanide
Specific Gravity
Oil and Grease/Total Petroleum Hydrocarbons
Acid Volatile Sulfides (AVS)
Total Sulfides
Total Kjeldahl Nitrogen (TKN)
Salinity (interstitial water)
Ammonia (interstitial water)
Sulfides (interstitial water)

Decisions concerning which parameters to measure are generally driven by program needs, historical data, or a "reason to believe." District personnel should coordinate these analyses with other regulatory agencies.

Health and safety issues should be discussed not only in SOPs, but also in written toxicological testing procedures.

4 Sample Handling, Storage, and Shipment

Sample handling, storage, and shipment procedures are used for transferring sample custody, compositing samples, storing samples, and coordinating the final disposition of samples. Consistency in sample handling and tracking is important because decisions having possible legal ramifications may be made on the results of analyses of sediment samples. To be able to make sound decisions and for results to be scientifically defensible, it is essential that these samples can be traced back to their source. Four major issues related to sample handling were discussed during the QA/QC Workshop:

a. Internal chain-of-custody.

b. Sample sieving.

c. Sample subdividing, homogenization, and compositing.

d. Sample storage and monitoring.

Internal Chain-of-Custody

Tracking procedures must demonstrate that the sample that was collected is the sample that was tested. This should be accomplished through the use of standard sample tracking (chain-of-custody) forms. Examples of these forms are presented in Appendix D.

Sample Sieving

Recommendations regarding sieving of test material prior to testing are prescribed in the test protocols. If sieving is required, all sediments (including reference, control, and test) should be press sieved prior to testing. In most cases, a 0.5-mm screen size is sufficient for removing predators and competitors.

Sample Subdividing, Homogenization, and Compositing

Sediments should be homogenized to consistent color and texture prior to testing. Clean, noncontaminating containers and implements should be used to handle and store sediments. Suggested materials are stainless steel, Teflon, or Lexan. These containers may be specified in toxicological testing methods.

Sample Storage and Monitoring

Sediments must be stored at 4 °C and tested within 6 weeks from collection date (preferably 2 weeks). Whenever possible, samples should be stored with zero headspace or under nitrogen gas. Samples must be rehomogenized just prior to testing.

5 Data Recording, Reduction, Validation, and Reporting

Guidance in recording, reducing, validating, and reporting data is necessary to produce complete and scientifically defensible reports. District personnel must be explicit in what they expect to be in a report and the level of recording, reducing, and validation necessary to meet the data quality objectives. Examples of data analyses and reporting guidelines are presented in Appendix E. During the QA/QC Workshop, five major issues were discussed:

a. Use of laboratory notebooks, no-data entries, abbreviations, and corrections.

b. Data management, reporting, and validation procedures.

c. Identifying and handling unacceptable data and/or outliers.

d. Measurements of precision and accuracy.

e. Measurements of completeness and comparability.

Use of Laboratory Notebooks, No-Data Entries, Abbreviations, and Corrections

Standardized data recording forms and entry formats facilitate electronic transfer and manipulation of data. At a minimum, procedures for intra-laboratory data entry should be standardized. No-data entries should be marked with a "-" to indicate that data was not omitted. Abbreviations for technicians' names and routine laboratory observations are useful in reducing data recording and entry costs, but should be standardized whenever possible. A list of definitions should accompany data sheets and project files. Data should be recorded in indelible ink; corrections should be made by making a single line through the mistake, correcting the mistake, and dating and

initialing the correction. An initialed explanation for the lined-out data should be footnoted at the bottom of the data sheet.

Data Management, Reporting, and Validation Procedures

Standardization

Standardization of statistical analyses is strongly recommended. Consistency in data entry, analyses, and transfer will result in cost-effective laboratory efforts. Numerous toxicological databases are in existence, but a final decision on standardized data entry, analyses, and reporting procedures has not yet been made by regulatory agencies. QA/QC Workshop attendees recommended that criteria for standardization should be formulated as quickly as possible to reduce the number of potential standard procedures that might be developed by agencies. It was also recommended that further discussion on this topic should occur at conferences, such as those conducted by the American Society for Testing and Materials (ASTM) or Society of Environmental Toxicology and Chemistry (SETAC). In the interim, District personnel should attempt to standardize data entry and analyses as much as possible.

Validation

Original data should be validated (100 percent) at each level of transcription (e.g., entering data from bound laboratory notebooks into computer data bases). Upper level data validation (senior scientist/program manager) should be conducted on a minimum of 10 percent of the data points. External QA/QC review should be performed on a minimum of 10 percent of the data points. In addition, daily review should be conducted on all data forms for outliers or unusual observations. It was also suggested at the workshop that it would be helpful to include acceptable limits on data forms to ensure that outliers are identified early. At the end of a toxicity test, 10 percent of the test end points should be verified by another observer. Animals placed in exposure containers should be double counted to ensure that the correct number is tested.

Archival

Data storage and archival is program specific. Contractor archival requirements may vary from 5 to 20 years. Backup copies of all data should be maintained at a separate location.

Identifying and Handling Unacceptable Data and/or Outliers

Criteria for establishing outlier values are program specific. Toxicological testing end point outliers are generally more important than water quality outliers. Depending upon program requirements, outliers may be accepted and identified or rejected and selectively removed. Data may be analyzed with and without outliers. It was noted at the QA/QC Workshop that if the reason for an outlier can be explained, it can generally be removed from a data set. Outliers removed from a data set must be reported and the reasons for their removal justified.

Measurements of Precision and Accuracy

Laboratory precision for toxicological tests is concerned with the reproducibility of results under a given set of conditions. Accuracy is the measurement of the bias in the measurement system. During the QA/QC Workshop, it was suggested that measurements of precision could be obtained through the use of a negative control (native sediment) and through the use of standard reference toxicants. Laboratories could then evaluate standard reference toxicant results using a control chart and demonstrate appropriate testing procedures through acceptable negative sediment control survival. There was no consensus among the workshop participants on an appropriate measure for accuracy of these tests.

Measurements of Completeness and Comparability

Completeness is a measure of the amount of data obtained versus the amount of data originally intended to be collected. Most QA/QC Workshop attendees agreed that although 80- to 90-percent data completeness is usually acceptable, some studies may require a higher level of completeness to ensure confidence in data-analyses results. Completeness should be measured relative to whether data can be used with 100-percent confidence to make an environmental decision. Generally, end point data (e.g., survival, growth, and reproduction) should be 100-percent complete or the statistical power of the tests may be compromised. If data completeness is less than 80 percent for a toxicological test, that test may have to be repeated or best professional judgment used to assess the usefulness of the data for decision-making purposes. Comparability is defined as the confidence with which one data set can be compared with another. Comparability and confidence can be enhanced through interlaboratory calibration, the consistent use of one type of reference toxicant, and the use of intralaboratory control charts to assess test organism sensitivity.

6 Internal Quality Control Checks

Internal Quality Control Checks are used to determine whether toxicological tests are conducted in an appropriate manner. The frequency of these checks and the appropriate level of associated documentation is project specific. Quality control checks associated with toxicological tests might include examination documentation to ensure that all samples are tested, that sediment holding times are met, that holding conditions are acceptable, that survival in control sediment or water is appropriate, and that water quality is measured and within acceptable ranges. Examples of forms used in internal quality control checks are presented in Appendix F. During the QA/QC Workshop, three main issues associated with this topic were discussed:

a. Taxonomic verification and test organism handling.

b. Test validation controls and acceptable survival.

c. Reference toxicant and/or standard reference material testing.

Taxonomic Verification and Test Organism Handling

Since taxonomic verification requires qualified experts (whose opinions may differ), reference toxicant response should be considered as the primary means of assessing test organism appropriateness. The source of test organisms should be documented for each toxicological test as well as the response to reference toxicants. If possible, a subsample of the test organisms should be preserved for future identification if toxicological testing information is scattered or if reference toxicant results indicate a change in sensitivity. The age of organisms used for testing is usually specified in the protocols and, if possible, should be documented for each toxicological test. If age cannot be determined, the mean size or biomass at testing time should be documented. Test-organism loading rates are also generally determined by the testing protocol. Verification of loading rates via double counting is necessary as an internal quality control check.

Test Validation Controls and Acceptable Survival

Appropriate holding times and acclimation procedures should be specified in test protocols or SOPs and the resulting documentation made available for audit. At a minimum, laboratory seawater should be capable of supporting test organisms at a minimum of 90-percent survival for most toxicological tests. Other test-validation controls may be stipulated in specific testing protocols.

Reference Toxicant Testing

Reference toxicants should be used to assess test organism sensitivity. Results should be evaluated by developing a control chart for an LC_{50} response. A variety of techniques are used to construct and evaluate control charts. One technique is discussed in Chapter 3 of this document under Good Laboratory Practices. If specific guidance concerning the use of control charts is not available, an acceptable reference-toxicant response should be within two standard deviations of the mean control chart response.

Monitoring for Potential Laboratory Contamination

Laboratory water should be checked annually (more often, if necessary) for trace contaminants, and this data should be made available for audit. In addition, when appropriate, test-organism food and the tissues of test organisms held in culture should also be analyzed periodically for the presence of trace contaminants.

7 Corrective Action

Corrective action may be required when a deficiency or deviation from planning documents or procedures is discovered or when there are deviations from established Data Quality Objectives (DQOs). Deviations from planning documents should be documented on a deviation form. An example of this form is presented in Appendix G. District personnel must provide clear guidelines defining deviations, deficiencies, and appropriate corrective action when required. Three major topics were discussed at the QA/QC Workshop:

a. Data completeness.

b. DQO exceedences.

c. Techniques for corrective action.

Data Completeness

Data needs to be complete enough to enable decisions to be made (see section on Data Reporting). Minimum requirements should be set to allow for this. Although project specific, in general, 80- to 90-percent completeness is considered acceptable. It should be noted that there are different levels of importance associated with different categories of information (e.g., end point data is more important than water quality information; see section entitled Measurements of Completeness and Comparability in Chapter 5).

DQO Deviations

Deviations are defined as data that are outside of ranges specified in project DQOs. Out-of-compliance data may be due to deviations in test protocols or deficiencies associated with toxicological tests. Examples of DQO deviations in biological tests may include any of the following:

a. Excessive test organism mortality in control exposures.

b. Out-of-range water quality parameters.

c. Lack of randomization.

d. Lack of required reference, control, or reference toxicant exposures.

e. Out-of-range reference toxicant results.

Poor control survival, loss of control of exposure conditions, major mechanical errors, or mishandling of test organisms may result in a decision to retest; brief episodes of out-of-range water quality conditions, incomplete test monitoring information, or broken or misplaced test containers may only require that data be flagged and qualified.

A summary of typical test deviations and suggested corrective action is presented in Table 3.

Techniques for Corrective Action

Corrective actions relative to toxicological tests may include, but are not limited to, review of data and calculations, flagging and/or qualification of suspect data, or possible retesting. A review that provides a preliminary check of all "out of limit" events should be performed as soon as the data for a given parameter or test is tabulated and verified for accuracy. If there is any concern over the number of "out of limit" events, the contractor and District personnel should meet to decide what corrective action is required and whether retesting is necessary. Ideally, circumstances dictating retesting and who bears the cost associated with retesting should be spelled out in advance through the use of an indemnification contract such as that shown in Appendix B.

Table 3
Summary of Test Deviations and Suggested Responses

Deviation	Suggested Response	
	Retesting Required	Retesting May Be Required[1]
Lack of test array randomization		✓
Testing was not blind		✓
Required references or controls were not tested	✓	
Test chambers not identical		✓
Test container(s) broken or misplaced		✓
Test organism mortality in controls exceeds acceptable limits	✓	
Excessive test organism mortality in a single replicate of a control		✓
Test organisms were not randomly assigned to test chambers		✓
Test organisms were not from the same population		✓
Test organisms were not all the same species (or species complex)	✓	
Test organism holding times were exceeded		✓
Water quality parameters consistently out of range	✓	
Brief episodes of out-of-range water quality parameters		✓
Test monitoring was not documented		✓
Test monitoring was incomplete		✓
Sediment holding times were exceeded	✓[2]	
Sediment storage conditions were out of acceptable ranges		✓[2]

[1] If not retested, data may have to be qualified.
[2] Unless evidence is provided to show that sediment quality (geochemistry and contaminant levels) has not been affected.

8 Additional Recommendations

During the QA/QC Workshop, other recommendations were offered relative to QA/QC programs. These recommendations are summarized below:

a. Districts need guidance relative to which toxicological tests are appropriate to answer a particular question.

b. Early coordination with regulatory agencies relative to data quality objectives is essential to completing a project in a timely fashion.

c. All plans and SOPs should be approved prior to the start of work.

d. Compositing efficiency should be evaluated, if possible, by analyzing replicate chemistry samples.

e. In some instances, performance criteria should be applied to reference sediment.

f. A system for ensuring accurate identification of test organisms, the development of a standard reference sediment material, and the examination and compilation of existing positive and negative control results should be established. This will enable interlaboratory comparisons to be conducted.

g. Regulatory agencies and Districts need to more fully understand the limitations associated with toxicological tests. By understanding the application of each test, agencies and Districts will be better able to select the appropriate test to answer a particular question.

h. Additional guidance on setting data quality objectives should be given to Districts to ensure that the data generated is appropriate and useful for decision making.

i. This and other guidance documents should be made available to all agencies, Districts, and laboratories and periodically updated when new data become available.

j. Health and safety issues should be discussed not only in SOPs, but also in written toxicological testing procedures.

References

Battelle. (1992). *Standard methods manual for environmental sampling and analysis in San Francisco Bay.* Volumes 1-3. Draft Manual prepared for the U.S. Army Corps of Engineers under a Related Services Agreement with the U.S. Department of Energy in support of the Long-Term Management Strategy Program for San Francisco Bay, by Battelle, Pacific Northwest Laboratory, Richland, WA.

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U.S. Environmental Protection Agency/U.S. Army Corps of Engineers. (1991). *Evaluation of dredged material for ocean disposal - testing manual.* Prepared by the Environmental Protection Agency, Office of Water, Washington, DC, and Department of the Army, United States Army Corps of Engineers, Washington, DC, EPA-503/8-91/001, February 1991.

_____. (1993). *Evaluation of dredged material proposed for discharge in inland and near coastal waters - testing manual (draft). Inland testing manual.* Prepared by the Environmental Protection Agency, Office of Water and Office of Science and Technology, Washington, DC, and Department of the Army, United States Army Corps of Engineers, Washington DC, May 1993.

Appendix A
Workshop Attendees

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Appendix B
Contract Examples[1]

[1] References cited in this appendix are located at the end of the main text.

SECTION 27: LABORATORY TESTING WARRANTY/INDEMNIFICATION
(ADDENDUM TO STANDARD TERMS AND CONDITIONS)

DEFINITIONS:

Seller (Laboratory) by formal acknowledgement and/or by performance to the Statement of Work (if applicable) under this order agrees to the following sediment/biological testing warranty/indemnification scenarios which supercede all provisions of Section 6, Warranty insofar as where they conflict with the provisions of Section 27. Where no conflict exists, Section 6, Warranty shall remain in effect as stated. For the purposes of Section 27 "Laboratory Performance Error" shall refer to the Seller's failure for reasons within control of the Seller to follow agreed upon test protocols. In addition, "Test Species Failure" shall refer to the failure of the Test Species to perform for reasons beyond the control of the Seller when the Seller has followed all agreed upon test protocols.

PROVISIONS:

A. Seller agrees to conduct a second test if Buyer has provided sufficient sample volume and adequate time for sample retest after a laboratory performance error or test species performance failure has rendered an initial test invalid. Seller will be paid only for the second test (if properly conducted). No payment by buyer will be made for the initial test. Seller is responsible for test species performance. Failure to achieve acceptable performance criteria due to test species failures will result in no payment to Seller for the unacceptable test.

B. (Reference attached Page 3 of 3 "Laboratory Testing Warranty/Indemnification Matrix"):

Seller agrees (in concert with attached Matrix) if Buyer has provided sufficient sample volume and adequate time for a sample retest, Seller is obligated to re-perform the failed test under a lab performance error or a species failure (subject to the conditions within Paragraph E). Should Seller experience a laboratory performance error in either the first test or any subsequent retests regardless of test species failure resulting in invalid data as determined by the cognizant Federal or State agency, Seller shall indemnify Buyer and assumes full responsibility and liability for further sample collection and test costs which are reasonable and necessary to allow retesting. Buyer shall charge Seller at the prevailing rates, incurred at time of resampling and retesting.

1 of 3

Figure B1. Example of indemnification statement (Science Applications International Corporation (SAIC) 1993) (Sheet 1 of 3)

C. Should holding times or sample volume requirements allow only one laboratory test, no payment by Buyer will be required in the event of a laboratory performance error under these circumstances. Buyer will not invoice its client for testing services, but there will be no liability upon Seller for resampling. There will also be no liability upon Seller for resampling costs in the event of a test species failure, or other conditions beyond the control of the Seller which result in a test failure.

D. In the event a Seller's test is judged invalid as determined by the agency for which the tests are submitted, whether for laboratory performance error or for test species performance failure, the Buyer will not be liable for payment for those tests.

E. The Seller may make a determination prior to initiating any tests, that physical and biological conditions prevailing at the time of the test, may result in test species performance failure. The Seller has the option of informing the Buyer of these conditions, and not proceeding with the tests. Seller will not indemnify Buyer for not conducting said tests. Buyer then has the option of negotiating with its client on a change in testing requirements, or contracting with a third party for completion of the biological tests.

F. The above (A through E) provisions do not waive Buyer's or Seller's rights under any other clause of Buyer's Standard Terms and Conditions of this order unless otherwise indicated above. For purposes of Lab Testing, indemnification and consequential damages shall be limited to the costs of retesting, resampling/collection in accordance with prevailing rates as applicable.

Figure B1. (Sheet 2 of 3)

LABORATORY TESTING WARRANTY/INDEMNIFICATION. CONDITIONS OF PAYMENT, NON-PAYMENT, AND INDEMNIFICATION.

SCENARIO	TEST 1	TEST 2	CONDITION (Refer Below)
1	Successful test		Payment (1)
2	Species Failure	Insufficient sample or time	No payment (2)
3	Lab Performance Error	Insufficient sample or time	No payment (2)
4	Species Failure	Successful Test	Payment (1)
5	Lab Performance Error	Successful Test	Payment (1)
6	Species Failure	Species Failure	No Payment (2)
7	Species Failure	Lab Performance Error	Indemnification (3)
8	Lab Performance Error	Lab Performance Error	Indemnification (3)
9	Lab Performance Error	Species Failure	Indemnification (3)

Condition Parameters:

(1). Payment for the successful test shall be made by Buyer to Seller.

(2) No payment for testing shall be made by Buyer to Seller. The Seller, however, shall not be held liable by Buyer for indemnification and consequential damages, as defined in Section 27 (F).

(3) No payment for testing shall be made by Buyer to Seller. The Seller shall indemnify Buyer as defined in Section 27 (F).

Figure B1. (Sheet 3 of 3)

STATEMENT OF WORK

January 22, 1992

PSDDA FULL CHARACTERIZATION OF THE LOWER SNOHOMISH RIVER CHANNEL

INTRODUCTION

The U.S. Army Corps of Engineers plans to conduct maintenance dredging in the lower Snohomish River channel and has performed a partial sediment characterization in order to reassess the ranking of the dredging area. Based on the results of the partial characterization, a full sediment characterization was performed, and involved the collection of 28 sediment cores from depths of approximately 4 to 12 feet. The cores were composited into 12 sediment samples for chemical analysis in accordance with current PSEP/PSDDA protocols. Based on the results of the chemistry analysis, 3 samples were identified for toxicity testing.

SCOPE OF SERVICES

will test 5 marine sediment samples (3 test and 2 reference sediments) using the following PSDDA bioassays:

- 10-day marine amphipod acute toxicity test using *Rhepoxynius abronius*
- Sediment larval bioassay using *Dendraster excentricus*
- Microtox test (saline extract)
- 20-day *Neanthes* biomass test using *Neanthes arenaceodentata*

Samples will be analyzed in accordance with the latest PSDDA (1989) and PSEP (1986, 1991) guidelines included in the following documentation:

- Recommended Protocols for Conducting Laboratory Bioassays on Puget Sound Sediments (PSEP), July 1991;
- Management Plan Report, Unconfined Open-Water Disposal of Dredged Material, Phase II. In Puget Sound Dredged Disposal Analysis Reports - September 1989 and revisions made on February 2, 1990;
- Modifications as specified by PSDDA during public workshops and the annual review process.

Specific . protocols that may be used for these characterizations are referenced in the Quality Assurance Program Plan submitted to dated May 16, 1991. These protocols are numbered as:

- #9020. *Rhepoxynius abronius* bedded sediment test
- #9022 *Dendraster excentricus* sediment larval test
- #9024 *Neanthes* 20 day chronic

Figure B2. Example of statement of work (SAIC 1993) (Continued)

Appropriate PSDDA/PSEP quality control analyses will be conducted for all bioassays. West Beach sediment will be used as both a negative control and reference for the two coarser samples, C4 and C11, which had 15 and 12 percent fines, respectively. A reference sediment collected from Carr Inlet and provided by will be used for C8 (48% fines). Appropriate water quality monitoring will be conducted for amphipod, sediment larval, and *Neanthes* bioassays (pH, salinity, temperature, dissolved oxygen). Ammonia and sulfides will be determined at test initiation and termination for the amphipod and echinoderm tests and at test initiation for the *Neanthes* test. Interstitial salinity will be determined prior to test set up. A QA2 data package is also required and will be submitted with the final data reports.

Aeration during the tests and sieving of the West Beach reference sediment is currently under discussion. Directions for aeration and sieving will be communicated to prior to test initiation.

DELIVERABLES

The full characterization bioassay results will be delivered within 35 days following receipt of the samples. The data reporting will follow PSDDA/PSEP guidelines.

Samples will be referenced to specific analytical batches which include the identification and extraction/analysis date of corresponding blank samples, reference sediment, and other QC sample data.

SCHEDULE

Holding times for the sediment samples commenced December 14, 1992. will collect the West Beach reference sediment during collection of the amphipods and control sediment. Enough reference sediment will be collected by to perform bioassay tests and PSDDA conventional analyses. PSDDA conventional analyses (including sample jars) will be arranged by Carr Inlet reference sediments will be provided by Sample delivery to is expected to occur during the week of January 25-29.

will provide the final data report 35 calendar days after receipt of the samples.

Figure B2. (Concluded)

Appendix C
Standardized Testing Forms and Checklists[1]

[1] References cited in this appendix are located at the end of the main text.

QUALITY CONTROL CHECKLIST FOR 10-DAY AMPHIPOD TEST

SPECIES: (check one)

Rhepoxynius abronius _____
Ampelisca abdita _____

PROJECT DATA

PROJECT NAME: _____

PROJECT NUMBER: _____

CLIENT: _____

CLIENT CONTACT: _____

ADRESS _____

_____

_____

_____

PHONE NUMBER _____

PROTOCOL

Project Testing Program (check one)

PSDDA _____
PSEP _____
Green Book _____
Other _____

Laboratory Protocol Number _____
Protocol Reviewed and Signed by Client? _____

PROJECT STAFF

Principal Investigator _____
Associate Investigator _____
Staff _____

_____

_____

_____

QA Officer _____

Protocol Reviewed by all project staff? _____

Figure C1. Quality control checklist for 10-day amphipod test (Science Applications International Corporation (SAIC) 1993)

PROJECT SCHEDULE

Sediment Collection and Expiration Dates

Date of First Sediment Collection _____

Date Sediment Delivered _____

Holding Time (check one) 2 weeks _____

8 weeks _____

Holding Time Expiration _____

Amphipod Collection and Handling Conditions

Date of Amphipod Collection: _____

Site of Amphipod Collection _____

Field Personnel _____

Collection site salinity _____

Collection site temperature _____

Field weather conditions _____

Time initiated - time completed _____

Time Arrive Lab: _____

Water Temperature at return _____

Storage Facility: _____

Storage Temp: _____

Buckets Aerated: _____

Field Notes: _____

_____

_____

_____

_____

Exposure Dates

Test Setup _____

Amphipod Innoculation _____

Test Breakdown _____

Reporting Requirements

Data available for report compilation _____

Draft Report completed _____

QA Review by: _____

Report Due to Client By: _____

Figure C2. Project schedule form (SAIC 1993)

AMPHIPOD SETUP PROCEDURAL CHECKLIST

Seawater Collection, Filtration, Preparation

Seawater Volume Required	_____
Approximate Volume Collected	_____
Date and Time of Collection	_____
Location of Source Water	_____
Collection Temperature	_____
Filter and Adjust Sea water	_____
Final Seawater Salinity (o/oo)	_____

Randomization Schedule

Randomization prepared by	_____
Place copy of schedule with this file	_____

Sediment Setup

Measure and record interstitial salinity	_____
Sieve control sediment and wash with clean sea water. Use .5mm screen.	_____
Verify temperature of water bath or E.C.	_____
Check to see that the light cycle is set for constant illumination.	_____
Use deconed stainless steel spoons and plasitc cups to dispense 175ml of sediment into each test vessel .	_____
Take ammonia and sulfide for each station	_____
Purge remaining sample containers with Nitrogen	_____
Add adjusted seawater to test vessel to 1000 mL	_____
Aerate all replicates @ < 100 bubbles/minute	_____
Put glass covers on all replicates	_____
Clean up the lab area	_____
Initials of individual verifying completion of tasks	_____

Figure C3. Amphipod setup procedural checklist (SAIC 1993)

Collect amphipods by sieving a small amount of sand through a 1.0 mm NITEX screen in seawater. Take screen out of water for just a moment, and then place back into the seawater. The amphipods will float, and are available for collection with a glass or plastic beaker.

Sort 10 sexually immature amphipods approximately 4mm into a plastic cup, with 1/4 in. of seawater and keep on ice. DO NOT collect obvious females with brood pouch, or sexually active males.

QC Amphipods with dissecting microscope. DO NOT use Stage Lighting System. _____

QA counts in cups prior to innoculation. _____

Monitor physical parameters (DO, pH, salinity) in all vesels prior to innoculation. _____

Combine Amphipods into groups of 20 placing the empty cup on the bottom of the full cup. Being careful not to leave any in the empty cups. _____

To seed Amphipods first remove all the watch glasses from one row of test vessels. Next seed the vessel farthest away, Check to see that all amphipods sink into water column and are not retained in the medicine cup. replace the watch glass and place the empty medicine cup on top of it. Proceed to the next test vessel

Seed reference toxicant replicates _____

Allow Amphipods one hour to rebury in test sediment if they do not bury. _____

Remove them using a clean pippet and replace them with a healthy amphipod _____

Check to make sure Ref-Tox Amphipods are not trapped on surface _____

Take Ammonia and Sulfide samples. _____

Each label should contain date, time, organism, Sulfide or Ammonia, test name and number, sample name or number, and initials. _____

Check to make sure watch glasses are placed on ref-tox and amphipods are not trapped on surface _____

Refrigerate Ammonia and Sulfide sample bottles _____

Clean laboratory area _____

Initials of individual verifying completion of tasks _____

Figure C4. Amphipod inoculation procedure (SAIC 1993)

Amphipod Reference Toxicant Procedures

For use with *Rhepoxynius abronius & Ampelisca abdita*

Reference toxicant should be prepared as follows:

Positive control: Cadmium Chloride. Express concentrations as Cd.
Stock Solution prepared at 10 mg/L
Stock Preparation Date:_____

Preparation of Reference Toxicant Replicates

Cd Concentation	ml Stock Solution	ml Seawater	Label Replicates
1.5mg/l	0.15	999.85	A - C
.75mg/l	0.075	999.925	D - F
.25mg/l	0.025	999.975	G - I
0.0mg/l	0	1000	J - K

Preserve 100 mL of Reference Toxicant at highest concentration for analysis _____

Reference Toxicant Replicates Prepared By: _____

Figure C5. Amphipod reference toxicant procedures (SAIC 1993)

AMPHIPOD SETUP FORM

Project _____ Test Organism _____
Project Number _____
Date: _____ Page ____ of ____

Project Sample I.D. Number	Lab Replicate Number	Interstitial Salinity	Soil Type/Comments	Initials

Figure C6. Amphipod setup form (SAIC 1993)

AMPHIPOD DAILY QC CHECKLIST

Project _____
Project Number _____

Conduct task, and initial after completion

Date:	Day 0	Day 1	Day 2	Day 3
Monitor	___	___	___	___
Emergence	___	___	___	___
Note observations	___	___	___	___
Monitor Ref-Tox	___	___	___	___
Check aeration	___	___	___	___
Restore water levels with D.I. water	___	___	___	___

Date:	Day 4	Day 5	Day 6	Day 7
Monitor	___	___	___	___
Emergence	___	___	___	___
Note observations	___	___	___	___
Monitor Ref-Tox	___			
Check aeration	___	___	___	___
Restore water levels with D.I. water	___	___	___	___
Replace Temp. Record Card				___
Ref Tox Breakdown	___			

Date:	Day 8	Day 9	Day 10
Monitor	___	___	___
Emergence	___	___	___
Note observations	___	___	___
Check aeration	___	___	___
Restore water levels with D.I. water	___	___	___
Remove Temp. Record	___	___	___

Figure C7. Amphipod daily QC checklist (SAIC 1993)

AMPHIPOD SOLID PHASE BIOASSAY: DAILY OBSERVATION FORM

Project ____________________ Test Start ____________

Project Number ____________________ Test End ____________ Page ______ of ______

	Amphipods Visible									
Replicate	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10
Initials										

Figure C8. Amphipod solid-phase bioassay: Daily observation form (SAIC 1993)

AMPHIPOD DAILY WATER QUALITY MONITORING

Project _____ Test Organism _____
Project Number _____ Test Day # _____
Page ____ of ____ Date _____

Instrument Serial Number _____

Replicate	Temperature (degrees-C)	pH	Salinity (o/oo)	D.O. (mg/L)

Figure C9. Amphipod daily water quality monitoring form (SAIC 1993)

AMPHIPOD BREAKDOWN PROCEDURAL CHECKLIST

Conduct Final Replicate Physical Monitoring _____

Record Daily Observations _____

Take ammonia and sulfide samples _____
(Each label should contain date, time, organism,
Sulfide or Ammonia, test nameand number, sample name
or number, and initials.)

Store ammonia and sulfide samples at 4 degrees C _____

Screen sediments using .5mm screen _____

Collect amphipods using a pipet and place in labelled medicine cup _____

Make sure Amphipods are kept in an adequate supply of sea water. _____

Place West Beach sand in medicine cups and add Amphipods. placing
empty Amphipod cup on the bottom. Leave for One Hour to rebury. _____

Record reburial data _____

Confirm all data is correctly entered, no blanks allowed. _____

File all raw data sheets with the project file, and copies with this notebook _____

Remove temperature record sheet and place in notebook _____

Clean laboratory after breakdown. _____

Schedule glass clean-up and decontamination _____

Initials of individual verifying completion of tasks _____

Figure C10. Amphipod breakdown procedural checklist (SAIC 1993)

AMPHIPOD BREAKDOWN DATA SHEET

Project ____________ Date ____________________

Project Number ____________ Page ______ of ______

Replicate	Initials	Visible	Found: Alive	Found: Dead	Reburied

Figure C11. Amphipod breakdown data sheet (SAIC 1993)

Appendix D
Chain-of-Custody Forms[1]

[1] References cited in this appendix are located at the end of the main text.

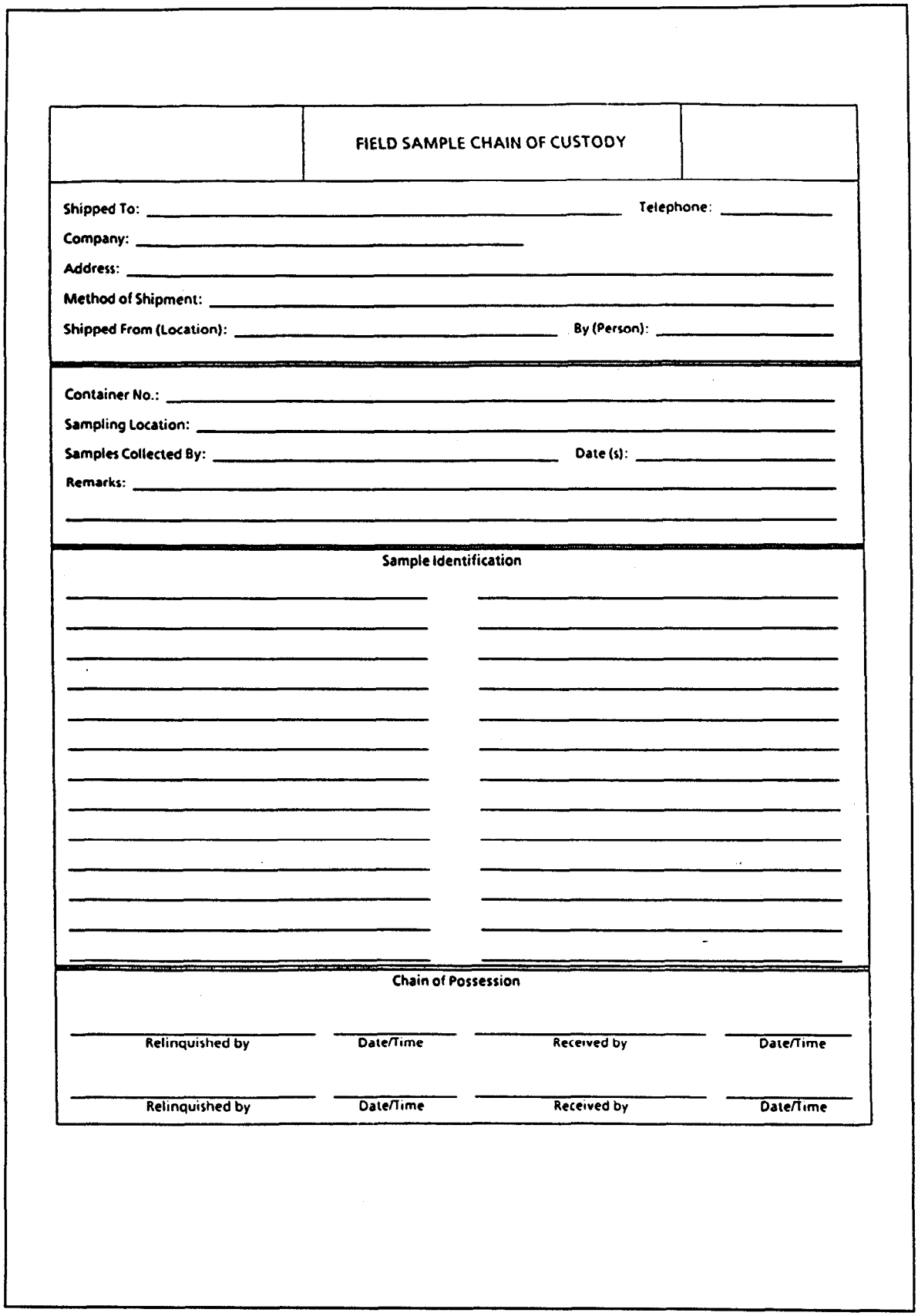

Figure D1. Field chain-of-custody form (Battelle 1992)

CHAIN-OF-CUSTODY RECORD

PROJECT:					SAMPLERS: *(Signature)*				
SAMPLE NO.	SITE	DATE	TIME	SAMPLE MATRIX			NO. CONTAINERS	REMARKS
				WATER	SEDIMENT	TISSUE		
RELINQUISHED BY: *(Signature)*				RECEIVED BY: *(Signature)*				DATE/TIME
RELINQUISHED BY: *(Signature)*				RECEIVED BY: *(Signature)*				DATE/TIME
RELINQUISHED BY: *(Signature)*				REC'V'D BY MOBILE LAB FOR FIELD ANAL: *(Signature)*				DATE/TIME
DISPATCHED BY: *(Signature)*			DATE/TIME		RECEIVED FOR LAB BY: *(Signature)*			
METHOD OF SHIPMENT:								

Figure D2. Laboratory chain-of-custody form (U.S. Environmental Protection Agency/U.S. Army Corps of Engineers 1993)

Appendix E
Examples of Data Analysis and Reporting Requirements[1]

[1] References cited in this appendix are located at the end of the main text.

4.0 DATA ANALYSIS AND TEST RESULT REPORTING

4.1 DATA ANALYSIS

A single-tailed t-test or Dunnet's Analysis of Variance will be used to compare the mortality in the seawater control to the mortality in the test sediments (Zar, 1984). Results of these tests will then be compared to the appropriate regulatory guideline (eg., PSDDA, Washington Sediment Guidelines).

Mortality data derived from the reference toxicants are used to generate test material response curves and statistically estimate an LC_{50} and its 95% confidence limit. The LC_{50} is the concentration of the test material which produces a mortality in 50% of the test population for the specified exposure duration. LC_{50} values will be based on the material concentrations. An LC_{50} will be estimated for the 4-day exposure duration.

A statistical computer package will be used to estimate LC_{50}s and their 95 % confidence limits. These programs estimate LC_{50} values using one of three statistical methods: probit analysis, moving average method, or binomial probability. The method selected is determined by the quality of the concentration-percent response base (i.e., presence or absence of 100% response, number of partial responses, etc.). The program provides values of the slope, for the probit analysis, includes 95% confidence intervals, and statistically evaluates the goodness-of-fit.

4.2 REPORTING

The results of the test will be presented in a report. The report will contain both raw data (i.e., logs showing responses of test specimens, water quality measurements) and summarized data. All values of chemical and water quality measurements are reported to various significance levels depending on the accuracy of the measurement devices employed.

All reports will include, but are not limited to information shown in the report outline (Table 2).

4.3 DATA RETENTION

All original raw data will be retained in archives, in a locked, fire-proof file cabinet.

Figure E1. Example of data analyses directions and reporting requirements (Science Applications International Corporation (SAIC) 1993)

Section	Description of Information
Title Page	
Summary of Test	Parametrix Inc. report and project numbers, test protocol, dates when the tests were conducted.
Study Specific Information	Address of the Performing Laboratory(ies) and site(s), and key personnel involved in the study, i.e., Quality Assurance Unit, Program Coordinator, Study Director, Principal Investigator.
Methods	Observations of appearance and behavior of the test material in the test chambers.
	Source, treatment and chemical analysis of dilution water.
	Source and collection technique of *R. abronius*.
	Description of biological observations, test endpoints, and chemical measurements, including basic water quality and analyses of the test material, if measured.
Results	Summary of water quality conditions existing during the tests.
	Summary of the responses of the test specimens to the test material.
	4-day LC_{50} for reference toxicant with 95% confidence limits.
Quality Assurance	Deviations from the protocol not addressed in protocol amendments will be listed, together with a discussion of the impact on the study and signed by the Study Director.
Appendices	Raw Data Protocols

Figure E2. Example outline for a toxicity testing report (SAIC 1993)

Appendix F
Data Quality Assurance Reviews[1]

[1] References cited in this appendix are located at the end of the main text.

CHECKLIST FOR AMPHIPOD MORTALITY BIOASSAY

Project Name: _____ Project No: _____

Laboratory: _____ Lab Number _____ Batch _____

Responsible Technician _____ Reviewed By: _____

Amphipod species _____

Date Sampled _____ Received by Lab _____

Date Analysis Begun _____

Problems noted (e.g., deviations from prescribed methods, analytical problems)

_____

_____

_____

_____

COMPLETENESS AND HOLDING CONDITIONS

Samples Submitted _____ # Samples Analyzed _____

Holding conditions acceptable (Y/N) PSEP ; 4° C under nitrogen < 2 weeks _____

PSDDA; 4° C under nitrogen < 8 weeks _____

If no, identify samples _____

_____

FORMAT

Standard data report sheet (check off)

Number of amphipods reported for each replicate	_____	Field samples	_____
Percent Mortality reported for each replicate	_____	Positive controls	_____
Daily emergence taken for each replicate	_____	Negative controls	_____
Individual replicate, plus sample mean and standard deviations for mortality?			_____

Analytical Replicates

Number per Sample _____

Any < 5 RPD? _____

Water Quality Variable Reported for each Replicate (check)

Interstitial salinity for each sample (initiation)	_____	Salinity (daily)	_____
Dissolved Oxygen (daily)	_____	pH (daily)	_____
Temperature (daily)	_____	Sulfide (initiation and termination)	_____
Ammonia (initiation and termination)	_____		

Figure F1. Checklist for amphipod mortality bioassay (Science Applications International Corporation (SAIC) 1993) (Continued)

CHECKLIST FOR AMPHIPOD MORTALITY BIOASSAY

QA/QC SAMPLES

Negative Control

Control Sediment Collection Site _____
Water Source _____
Current priority pollutant scan available? _____
Mean Control Mortality (%) _____
Exceed PSEP QA Limit of 10%? _____

Reference Sediment

Collection Site _____
Total Number of Analyses _____
Mean Mortality _____
Mean mortality exceed PSEP QA limit of > 20% over control? (Y/N) _____

Positive Controls

Reference Toxicant _____
Exposure Concentrations _____
% mortality/exposure concentration _____
Organism Response (LC50) _____
Laboratory Performance Standards for Reference Toxicant _____
Did the test LC50 fall within lab standards (Y/N)? _____

WATER QUALITY

Samples with temperature <14 or > 16° C _____
Samples with salinity < 27 or > 30 ppt _____
Samples with pH < 7 or > 8 _____
Samples with DO < 5 mg/L _____

Figure F1. (Concluded)

AMHIPOD FINAL QUALITY ASSURANCE CHECK

TEST # EXPER. #

CONTROL % SURVIVAL THIS TEST: PASSED FAILED

Reference Test Experiment #: Control % Surviv.: LC 50:

List any problems associated with this test:

_____

_____

_____

_____

_____

_____

CHECK OF DATA INPUT PAGES

_____ Present: Method Summary sheet Randomization sheet (1-2 pg), Breakdown sheets (1-2 pg), 10 Daily Data sheets, Physical Data sheets (1-2), Field/Culture sheet Holding Time table

_____ Breakdown sheet: verify that vials were recounted.
_____ Breakdown sheet: verify that live animals found during repick were added to the total live pods.
_____ Breakdown sheet: verify that live animals found at 24, 48, 72 hr were added to total of live pods found.
_____ Breakdown sheet: verify that any tubes found at start were included in the total number of animals per rep.
_____ Breakdown sheet: check to see if the sum of the number dead during test and the number found live exceeds 20.
_____ Breakdown sheet: verify that reps in which no animals were found contained no tubes, molts, or animals dead durin
_____ Breakdown sheet: make sure that QA'd sheet was signed.
_____ From Holding Time sheet, verify that experiment numbers, sample numbers, collection dates, and Day 0 dates were and signed.
_____ From 10 day data sheets, verify the test day numbers on which the physical data were taken.

CHECK OF DATA OUTPUT PAGES

_____ Present: Data Entry Pages (1-2), Summary Data Pages (2), Data Base Pages (1-2) Project Summary Page Stat pages (1-2)

_____ RAND file: From randomization sheet: verify test and experiment number.
_____ RAND file: From randomization sheet: verify jar numbers.
_____ RAND file: From randomization sheet: verify sample numbers.
_____ RAND file: From holding time table: verify days hold.
_____ RAND file: Make sure experiment number appears in footer.

_____ SORT file: Verify that RAND file was QA'd and signed before it was converted to a SORT file.
_____ SORT file: Verify that the filename has been changed to SORT.
_____ SORT file: From 10 day data sheets: Verify temperature range.
_____ SORT file: From breakdown sheet: verify that correct breakdown sheet was used.
_____ SORT file: From breakdown sheet: verify values for number alive (total live pods at end of test).
_____ SORT file: From breakdown sheet: verify values for the number of pods added to each jar (# per rep).
_____ SORT file: From physical data sheet: verif that correct physcl data sheet was used as a source of the pH. D O . Sali
_____ SORT file: verify that the physical data were entered for the correct two replicate numbers
_____ SORT file: verify pH values.
_____ SORT file: verify D.O. values.
_____ SORT file: verify salinity values.

_____ CALC file: Verify that SORT file was QA'd and signed before it was posted into CALC file.

_____ CALC file: verify that reps for which there is no data have an empty cell under decimal mortality.
_____ CALC file: Stats page(s): verify that reps for which there is no data have been replaced by a period.
_____ CALC file: Database pages: Verify that reps for which there is no data have been cleared.
_____ From Breakdown sheet: verify that comments have been transferred to Summary Data Pages.
_____ From 10 Day Data Sheets: verify that comments have been transferred to Summary data sheets.
_____ From Physical Data Sheets: verify that comments have been transferred to the Summary Data Sheets.
_____ From the corresponding Reference Test: verify that any comments have been transferred to the

Figure F2. Amphipod final quality assurance check (SAIC 1993)

CHECKLIST FOR SEDIMENT LARVAL BIOASSAY (SOLID PHASE)

Project Name: _____ SAIC Project No: _____

Laboratory: _____ Lab Number _____ Batch _____

Responsible Technician _____ Reviewed By: _____

Date Sampled _____ Received by Lab _____

Date Analysis Begun _____

Problems noted (e.g., deviations from prescribed methods, analytical problems)

_____

_____

_____

_____

COMPLETENESS AND HOLDING CONDITIONS

Samples Submitted _____ # Samples Analyzed _____

Holding conditions acceptable (Y/N) PSEP ; 4° C under nitrogen < 2 weeks _____
PSDDA; 4° C under nitrogen < 8 weeks _____

If no, identify samples _____

_____

FORMAT

Standard data report sheet (check off)

Number of larvae evaluated _____ Field samples _____
Percent Mortality reported for each replicate _____ Positive controls _____
Percent Abnormality reported for each replicate _____ Negative controls _____
Individual replicate, and sample mean and standard deviations for mortality and abnormality? _____

Water quality variable reported for each replicate
Dissolved Oxygen (daily) _____ Salinity (daily) _____
Temperature (daily) _____ pH (daily) _____
Ammonia (initiation and termination) _____ Sulfide (initiation and termination) _____

QA/QC SAMPLES

Negative Control (seawater)
Water Source _____
Current priority pollutant scan available? _____

Mean Control Mortality (%) _____ Exceed PSEP QA Limit of 50%? _____
Exceed PSDDA QA Limit of 30%? _____
Mean Control Abnormality (%) _____ Exceed PSEPE/PSDDA Limit of 10%? _____

Figure F3. Checklist for sediment larval bioassay (solid phase) (SAIC 1993) (Continued)

CHECKLIST FOR SEDIMENT LARVAL BIOASSAY (SOLID PHASE)

PAGE 2

Negative Control (sediment)
Collection Site
Mean Control Mortality (%)
Mean Control Abnormality (%)

Analytical Replicates
Number per Sample
Any < 5 RPD?

Positive Controls
Reference Toxicant
Exposure Concentrations
% mortality/exposure concentration
Organism Response (LC50)
Laboratory Performance Standards for Reference Toxicant
Did the test LC50 fall within lab standards (Y/N)?

Reference Sediment
Collection Site
Total Number of Analyses
Mean Mortality
Mean Abnormality
Mean mortality and abnormality > 20% over control? (Y/N)

WATER QUALITY

Dendraster excentricus
Samples with temperature <14 or > 16° C
Samples with salinity < 27 or > 29 ppt
Samples with pH < 7 or > 9
Samples with DO < 5 mg/L

Crassostrea gigas
Samples with temperature <19 or > 21° C
Samples with salinity < 27 or > 29 ppt
Samples with pH < 7 or > 9
Samples with DO < 5 mg/L

Mytilus spp.
Samples with temperature <15 or > 17° C
Samples with salinity < 27 or > 29 ppt
Samples with pH < 7 or > 9
Samples with DO < 5 mg/L

Strongtyocentrotus purpuratus
Samples with temperature <14 or > 16° C
Samples with salinity < 27 or > 29 ppt
Samples with pH < 7 or > 9
Samples with DO < 5 mg/L

Figure F3. (Concluded)

QA/QC CHECKLIST

_____ QA MANAGEMENT PROGRAM - *integrates management and technical practices; ensures project elements and activities comply with regulatory guidelines.*

_____ QA PROJECT PLAN - *Overall plan for activities performed at each stage of the dredged-material evaluation.*

_____ **Project description** - *Defines project goals and methods to achieve those goals.*

_____ **Project organization; personnel responsibilities and qualifications**

_____ Organizational flow diagrams and/or tables

_____ **Data quality objectives**

_____ Data quality objectives summary table

_____ **Sampling procedures**

_____ Sampling plan outlining all methods, procedures, and equipment

_____ Checklists for field equipment, sample container preparation, sample preservation, etc.

_____ Alteration checklist

_____ **Sample custody and documentation** - record all events associated with a sample

_____ Sample labels

_____ Tracking report forms (field and laboratory)

_____ Chain-of-custody forms

_____ Inventory log

_____ Record of field procedures

_____ Station location log

_____ **Calibration procedures**

_____ Procedures used to assure field and laboratory equipment are calibrated and functioning properly

_____ **Standard operating procedures** - *written descriptions of routine methods*

_____ **Data validation, reduction, and reporting** - *procedures used to accept or reject data after collection*

_____ **Chemical Quality Control** - *procedures to be used to demonstrate precision, accuracy, comparability, completeness, and representativeness*

_____ **Performance and system audits** -*an independent evaluation of field and laboratory procedures*

_____ Systems Audit Checklist

_____ **Facilities** - *a complete, detailed description of physical layout of laboratory with the areas defined where each test will be performed*

_____ **Preventative maintenance procedures and schedules** - *procedures and schedules to be used to ensure equipment will be maintained in proper working order*

Figure F4. Quality assurance/quality control checklist (U.S. Environmental Protection Agency/U.S. Army Corps of Engineers 1993) (Continued)

_____ Records of calibrations

_____ Records of corrective actions applied

_____ **Specific routine procedures to be used to assess data precision, accuracy, and completeness** - *calculations, equations, and type of samples to assess precision, accuracy and completeness of data*

_____ **Corrective action** - *identification of nonconformance events*

_____ Records of corrective actions applied

_____ Alteration checklist

_____ **Physical and chemical analyses** - *procedures to be used to ensure analyses of seawater, sediment, and tissues meet acceptable criteria*

_____ **Biological analyses** - *procedures to be used to ensure the condition of test animals and results of biological tests meet acceptable criteria*

_____ Reference Toxicant/Test Organism Control Charts

_____ **QA reports to management** - a description of methods to be used for periodic reporting to management

Figure F4. (Concluded)

Appendix G
Deviations and Corrective Action[1]

[1] References cited in this appendix are located at the end of the main text.

CORRECTIVE ACTIONS CHECKLIST

SAMPLE PROGRAM IDENTIFICATION: _____

SAMPLING DATES: _____

MATERIAL TO BE SAMPLED: _____

MEASUREMENT PARAMETER: _____

ACCEPTABLE DATA RANGE:

_____

CORRECTIVE ACTIONS INITIATED BY: _____

TITLE: _____

DATE: _____

PROBLEM AREAS REQUIRING CORRECTIVE ACTION: _____

_____

_____

_____

MEASURES TO CORRECT PROBLEMS: _____

_____

_____

MEANS OF DETECTING PROBLEMS (FIELD OBSERVATIONS, SYSTEMS AUDIT, ETC): _____

_____

_____

APPROVAL FOR CORRECTIVE ACTIONS: _____

TITLE: _____

SIGNATURE: _____

DATE: _____

Figure G1. Corrective action form (U.S. Environmental Protection Agency/U.S. Army Corps of Engineers (EPA/USACE) 1993)

ALTERATION CHECKLIST

Sample Program Identification: _____

Material to be Sampled: _____

Measurement Parameter: _____

Standard Procedure for Analysis: _____

_____

Reference: _____

_____

_____

Variation from Standard Procedure: _____

_____

_____

Reason for Variation: _____

_____

_____

Resultant Change in Field Sampling Procedure: _____

_____

_____

Special Equipment, Material, or Personnel Required: _____

_____

_____

Author's Name: _____ Date: _____

Approval: _____ Title: _____

Date: _____

Figure G2. Alteration checklist (EPA/USACE 1993)

Appendix H
Definitions

The following definitions used in this document are from the *Inland Testing Manual* (U.S. Environmental Protection Agency/U.S. Army Corps of Engineers 1993)[1] unless otherwise noted.

Accuracy	The ability to obtain a true value, determined by the degree of agreement between an observed value and an accepted reference value.
Acute Toxicity	Short-term toxicity to an organism(s) that has been affected by the properties of a substance such as contaminated sediment. The acute toxicity of a sediment is generally determined by quantifying the mortality of appropriately sensitive organisms that are put into contact with the sediment, under either field or laboratory conditions, for a specified period.
Audit	A planned and documented investigative evaluation of an item or process to determine the adequacy of and compliance with established procedures, instructions, Quality Assurance Project Plans, and other applicable documents (Battelle 1992).
Bioassay	A bioassay is an assay using a biological system. It involves exposing an organism to a test material and determining a response. There are two major types of bioassays differentiated by response: toxicity tests, which measure an effect (e.g., acute toxicity and sublethal/chronic toxicity), and bioaccumulation tests, which measure a phenomenon (e.g., the uptake of contaminants into tissues).

[1] References cited in this appendix are located at the end of the main text.

Comparability	The confidence with which one data set can be compared with others and the expression of results consistent with other organizations reporting similar data.
Completeness	A measure of the amount of data obtained versus the amount of data originally intended to be collected.
Control Sediment	A sediment essentially free of contaminants and compatible with the biological needs of the test organisms such that it has no discernible influence on the response being measured in the test. Control sediment may be the sediment from which the test organisms are collected or a laboratory sediment, provided the organisms meet control standards. Test procedures are conducted with the control sediment in the same way as the reference sediment and dredged material. The purpose of the control sediment is to confirm the biological acceptability of the test conditions and to help verify the health of the organisms during the test. Excessive mortality in the control sediment indicates a problem with the test conditions or organisms and can invalidate the results of the corresponding dredged material test.
Corrective Action	Activities required whenever a deficiency or deviation from planning documents or procedures is discovered or when there are departures from established Data Quality Objectives. These activities may include, but are not limited to, review of data and calculations, flagging of suspect data, or reanalyses of individual or entire batches of samples (Battelle 1992).
Data Quality Indicators	Quantitative statistics and qualitative descriptors that are used to interpret the degree of acceptability or utility of data to the user include bias (systematic error), precision, accuracy, comparability, completeness, and representativeness.
Data Quality Objectives	Qualitative and quantitative statements of the overall uncertainty that a decision maker is willing to accept in results or decisions derived from environmental data. DQOs provide the framework for planning environmental data operations consistent with the data user's needs.
Disposal Site	That portion of the "waters of the United States" where specific disposal activities are permitted and

	consist of a bottom surface area and any overlying volume of water.
District	A U.S. Army Corps of Engineers administrative area.
Dredged Material	Material that is excavated or dredged from waters of the United States.
EC_{50}	The median effective concentration. The concentration of a substance that causes a specified effect (generally, sublethal rather than acutely lethal) in 50 percent of the organisms tested in a laboratory toxicity test of specified duration.
Elutriate	Material prepared from the sediment dilution water and used for chemical analyses and toxicity testing.
Evaluation	The process of judging data in order to reach a decision.
Good Laboratory Practices	The minimum QA/QC requirements necessary in the performance toxicity tests. These requirements may include blind testing, randomization, minimization of cross-contamination, and the use of reference toxicants to assess test organism sensitivity.
Grain-Size Effects	Mortality or other effects in laboratory toxicity tests due to sediment granulometry, not sediment toxicity. It is clearly best to use test organisms that are not likely to react to grain size but, if this is not reasonably possible, then testing must account for any grain-size effects.
LC_{50}	The median lethal concentration. The concentration of a substance that kills 50 percent of the organisms tested in a laboratory toxicity test of specified duration.
Loading Density	The ratio of organism biomass or numbers to the volume of test solution in an exposure chamber.
Mixing Zone	A limited volume of water serving as a zone of initial dilution in the immediate vicinity of a discharge point where receiving water quality may not meet quality standards or other requirements otherwise applicable to the receiving water. (The mixing zone may be defined by the volume and/or the surface area of the disposal site or specific mixing zone definitions in State water quality standards.)

Precision	The ability to replicate a value; the degree to which observations or measurements of the same property, usually obtained under similar conditions, conform to themselves. Usually expressed as standard deviation, variance, or range.
QA	Quality assurance, the total integrated program for assuring the reliability of data. A system for integrating the quality planning, quality control, quality assessment, and quality improvement efforts to meet user requirements and defined standards of quality with a stated level of confidence.
QC	Quality control, the overall system of technical activities for obtaining prescribed standards of performance in the monitoring and measurement process to meet user requirements.
Reference Sediment	A sediment, substantially free from contaminants, that is as similar as practicable to the grain size of the dredged material and the sediment at the disposal site, and that reflects the conditions that would exist in the vicinity of the disposal site had no dredged-material disposal ever taken place, but had all other influences on sediment condition taken place.
Representativeness	The degree to which sample data depict an existing environmental condition; a measure of the total variability associated with sampling and measuring that includes the two major error components: systematic error (bias) and random error.
Sediment	Material, such as sand, silt, or clay, suspended in or settled on the bottom of a water body. The term dredged material refers to material that has been dredged from a water body, while the term sediment refers to material in a water body prior to the dredging process.
Standard Operating Procedure	A written document that details an operation, analysis, or action whose mechanisms are thoroughly prescribed and that is commonly accepted as the method for performing certain routine or repetitive tasks.
Testing	Specific procedures that generate biological, chemical, and/or physical data to be used in evaluations. The data are usually quantitative but may be qualitative (e.g., taste, odor, and organism behavior). Testing for

	discharges of dredged material in water of the United States is specified in 40 CFR 230.60 and 230.61.
Toxicity Test	A bioassay that measures an effect (e.g., acute toxicity and sublethal/chronic toxicity).
Whole Sediment	The sediment and interstitial waters of the proposed dredged material or reference sediment before it has undergone any process that may alter its chemical or toxicological properties.

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13. ABSTRACT *(Maximum 200 words)*

This report summarizes proceedings of a workshop on Quality Assurance and Quality Control (QA/QC) in laboratory bioassays of dredged material. The workshop was sponsored by the U.S. Army Engineer Waterways Experiment Station (WES). Attendees included individuals from academia, industry, and government with expertise in sediment toxicity testing and/or QA/QC. Topics included data quality objectives; biological procedures; sample handling storage and shipment; data recording, reduction, validation, and reporting; internal quality control checks; and corrective action. The report provides generic guidance under each of these topic headings. Appendices to the report include sample checklist, data reporting forms, chain-of-custody sheets, and laboratory testing contract indemnification forms.

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